

Amendment to the Claims

This listing of claims will replace all prior versions, and listing, of claims in the application.

Please amend the claims as follows:

Claim 1: (withdrawn) An isolated, synthetic or recombinant nucleic acid comprising

(a) a nucleic acid sequence having at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more or complete sequence identity to SEQ ID NO:41, over a region of at least about 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or more residues, wherein the nucleic acid encodes at least one polypeptide having a protease activity,

and optionally the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, and optionally the sequence comparison algorithm is a BLAST version 2.2.2 algorithm where a filtering setting is set to blastall -p blastp -d "nr pataa" -F F and all other options are set to default;

(b) a nucleic acid sequence encoding a polypeptide having a sequence as set forth in SEQ ID NO:42;

(c) a sequence that hybridizes under stringent conditions to a nucleic acid comprising SEQ ID NO:41, wherein the nucleic acid encodes a polypeptide having a protease activity,

wherein the stringent conditions comprise a wash step comprising a wash in 0.2X SSC at a temperature of about 65°C for about 15 minutes,

and optionally the nucleic acid is at least 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more residues in length or the full length of the gene or transcript; or

(d) a sequence complementary to (a), (b), or (c);

wherein optionally the protease activity comprises catalyzing hydrolysis of peptide bonds; comprises an endoprotease activity or an exoprotease activity; comprises a proteinase activity or a peptidase activity; comprises a carboxypeptidase activity; comprises an aminopeptidase activity; comprises a serine protease activity; comprises a metalloprotease activity; a matrix metalloprotease activity or a collagenase activity; comprises a cysteine protease activity; comprises an aspartic protease activity; comprises a chymotrypsin, a trypsin, an elastase, a kallikrein or a subtilisin activity; or, comprises a dipeptidylpeptidase activity,

wherein optionally the protease activity is thermostable or thermotolerant.

Claims 2 to 26 (canceled)

Claim 27 (withdrawn): A nucleic acid probe for identifying a nucleic acid encoding a polypeptide with a protease activity, wherein the probe comprises at least 10, or about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, about 60 to 100, or about 50 to 150, consecutive bases of

(a) a sequence comprising SEQ ID NO:41; or

(b) a sequence as set forth in claim 1; wherein the probe identifies the nucleic acid by binding or hybridization under stringent conditions,

wherein the stringent conditions comprise a wash step comprising a wash in 0.2X SSC at a temperature of about 65°C for about 15 minutes.

Claims 28 to 32 (canceled)

Claim 33 (withdrawn): An amplification primer pair, wherein the primer pair comprises a first member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more residues of

(a) a sequence as set forth in SEQ ID NO:41; or,

(b) a sequence as set forth in claim 1;

and a second member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more residues of the complementary strand of the first member.

Claims 34 to 39 (canceled)

Claim 40 (withdrawn): An expression cassette, a vector or a cloning vehicle comprising a nucleic acid comprising a sequence as set forth in Claim 1,

wherein optionally the cloning vehicle comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome,

and optionally the viral vector comprises an adenovirus vector, a retroviral vector or an adeno-associated viral vector,

and optionally the viral vector comprises an adenovirus vector, a retroviral vector or an adeno-associated viral vector,

and optionally the cloning vehicle comprises a bacterial artificial chromosome (BAC), a bacteriophage P1-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC).

Claims 41 to 44 (canceled)

Claim 45 (withdrawn): A transformed cell comprising a nucleic acid comprising a sequence as set forth in claim 1 or an expression cassette, a vector or a cloning vehicle as set forth in claim 40,

wherein optionally the cell is a bacteria cell, a mammalian cell, a fungal cell, a yeast cell, an insect cell or a plant cell.

Claims 46 to 47 (canceled)

Claim 48 (withdrawn): A transgenic non-human animal or transgenic plant or transgenic seed comprising a sequence as set forth in claim 1 or an expression cassette, a vector or a cloning vehicle as set forth in claim 40

wherein optionally the animal is a mouse;

wherein optionally the plant is a corn plant, a sorghum plant, a potato plant, a tomato plant, a wheat plant, an oilseed plant, a rapeseed plant, a soybean plant, a rice plant, a barley plant, a grass, a cottonseed, a palm, a sesame plant, a peanut plant, a sunflower plant or a tobacco plant;

wherein optionally the seed is a corn seed, a wheat kernel, an oilseed, a rapeseed, a soybean seed, a palm kernel, a sunflower seed, a sesame seed, a rice, a barley, a peanut, a cottonseed, a palm, a peanut, a sesame seed, a sunflower seed or a tobacco plant seed.

Claims 49 to 56 (canceled)

Claim 57 (withdrawn): A double-stranded inhibitory RNA (RNAi) molecule comprising a subsequence of a sequence as set forth in claim 1

wherein optionally the RNAi is about 15, 16, 17, 18 19 20, 21, 22 23, 24, 25 or more duplex nucleotides in length.

Claims 58 to 59 (canceled)

Claim 60 (currently amended): An isolated, synthetic, or recombinant polypeptide having at least ~~[[90%]]~~ 95% sequence identity to SEQ ID NO:42,

wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, and

wherein the polypeptide has a protease activity.

Claims 61 to 97 (canceled)

Claim 98 (previously presented): An array comprising an immobilized polypeptide as set forth in claim 60;

wherein the polypeptide is immobilized on a cell, a metal, a resin, a polymer, a ceramic, a glass, a microelectrode, a graphitic particle, a bead, a gel, a plate, an array or a capillary tube.

Claim 99 (canceled)

Claim 100 (withdrawn): An isolated or recombinant antibody that specifically binds to a polypeptide as set forth in claim 60 or encoded by a nucleic acid as set forth in claim 1;

wherein optionally the antibody is a monoclonal or a polyclonal antibody.

Claims 101 to 105 (canceled)

Claim 106 (withdrawn): A method of producing a recombinant polypeptide comprising the steps of: (a) providing a nucleic acid operably linked to a promoter, wherein the nucleic acid comprises a sequence as set forth in claim 1; and (b) expressing the nucleic acid of step (a) under conditions that allow expression of the polypeptide, thereby producing a recombinant polypeptide.

Claims 107 to 125 (canceled)

Claim 126 (withdrawn): A method for isolating or recovering a nucleic acid encoding a polypeptide with a protease activity from an environmental sample comprising the steps of:

(a) providing an amplification primer sequence pair as set forth in claim 33 or a polynucleotide probe comprising a sequence as set forth in claim 27;

(b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair or probe; and,

(c) combining the nucleic acid of step (b) with the amplification primer pair or probe of step (a) and amplifying or identifying a nucleic acid from the environmental sample, thereby isolating or recovering a nucleic acid encoding a polypeptide with a protease activity from an environmental sample,

and optionally the environmental sample comprises a water sample, a liquid sample, a soil sample, an air sample or a biological sample;

and optionally the biological sample is derived from a bacterial cell, a protozoan cell, an insect cell, a yeast cell, a plant cell, a fungal cell or a mammalian cell.

Claims 127 to 130 (canceled)

Claim 131 (withdrawn): A method of generating a variant of a nucleic acid encoding a polypeptide with a protease activity comprising the steps of:

(a) providing a template nucleic acid comprising a sequence as set forth in claim 1; and

(b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid;

optionally further comprising expressing the variant nucleic acid to generate a variant protease polypeptide;

optionally wherein the modifications, additions or deletions are introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSM), synthetic ligation reassembly (SLR) and a combination thereof;

optionally wherein the modifications, additions or deletions are introduced by a method comprising recombination, recursive sequence recombination, phosphothioate-

modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof;

optionally the method is iteratively repeated until a protease having an altered or different activity or an altered or different stability from that of a polypeptide encoded by the template nucleic acid is produced, and optionally the altered or different activity protease polypeptide is thermotolerant, and retains some activity after being exposed to an elevated temperature;

optionally the method is iteratively repeated until a protease coding sequence having an altered codon usage from that of the template nucleic acid is produced;

optionally the method is iteratively repeated until a protease gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced.

Claims 132 to 140 (canceled)

Claim 141 (withdrawn): A method for modifying codons in a nucleic acid encoding a polypeptide with a protease activity to increase its expression in a host cell, the method comprising the following steps:

- (a) providing a nucleic acid encoding a polypeptide with a protease activity comprising a sequence as set forth in claim 1; and,
- (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell, or, identifying a codon

in the nucleic acid of step (a) and replacing it with a different codon encoding the same amino acid as the replaced codon, thereby modifying codons in a nucleic acid encoding a protease, or, identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

Claims 142 to 172 (canceled)

Claim 173 (withdrawn): A method for hydrolyzing, breaking up or disrupting a protein-comprising composition comprising the following steps:

- (a) providing a polypeptide having a protease activity as set forth in claim 60;
- (b) providing a composition comprising a protein; and
- (c) contacting the polypeptide of step (a) with the composition of step (b)

under conditions wherein the protease hydrolyzes, breaks up or disrupts the protein-comprising composition;

optionally wherein the composition comprises a plant cell, a bacterial cell, a yeast cell, an insect cell, or an animal cell.

Claim 174 (withdrawn): A method for liquefying or removing a protein from a composition comprising the following steps:

- (a) providing a polypeptide having a protease activity as set forth in claim 60;
- (b) providing a composition comprising a protein; and
- (c) contacting the polypeptide of step (a) with the composition of step (b)

under conditions wherein the protease removes or liquefies the protein.

Claim 175 (previously presented): A detergent composition comprising the polypeptide of claim 60.



Claims 176 to 179 (canceled)

Claim 180 (previously presented): A textile or fabric comprising the polypeptide of claim 60.

Claims 181 to 184 (canceled)

Claim 185 (previously presented): A feed or a food comprising the polypeptide of claim 60.

Claims 186 to 189 (canceled)

Claim 190 (previously presented): A dairy product comprising the polypeptide of claim 60.

Claims 191 to 195 (canceled)

Claim 196 (previously presented): A paper or paper product or paper pulp comprising the protease of claim 60.

Claim 197 (canceled)

Claim 198 (previously presented): A pharmaceutical composition comprising the polypeptide of claim 60.

Claims 199 to 201 (canceled)

Claim 202 (previously presented): An oral care product comprising the polypeptide of claim 60,

wherein the product comprises a toothpaste, a dental cream, a gel or a tooth powder, an odontic, a mouth wash, a pre- or post-brushing rinse formulation, a chewing gum, a lozenge or a candy.

Claim 203 (canceled)

Claim 204 (previously presented): A contact lens cleaning composition comprising the polypeptide of claim 60.

Claim 205 (withdrawn): A method for treating solid or liquid animal waste products comprising the following steps:

- (a) providing a polypeptide as set forth in claim 60;
- (b) providing a solid or a liquid animal waste; and
- (c) contacting the polypeptide of step (a) and the solid or liquid waste of step (b) under conditions wherein the protease can treat the waste.

Claim 206 (withdrawn): A processed waste product comprising a polypeptide having a protease activity, wherein the polypeptide comprises a sequence as set forth in claim 60.

Claims 207 to 211 (canceled)

Claim 212 (previously presented): An antimicrobial, anti-viral or anti-spore agent comprising a polypeptide having a protease activity, wherein the polypeptide comprises the polypeptide of claim 60.

Claim 213 (previously presented): A disinfectant comprising a polypeptide having a protease activity, wherein the polypeptide comprises the polypeptide of claim 60.

Claim 214 (withdrawn): A method for tissue dissociation comprising the following steps:

- (a) providing a composition comprising a polypeptide having a protease activity as set forth in claim 60; and
- (b) contacting the composition of step (a) and with a tissue to be dissociated, wherein optionally the tissue is a wounded tissue, and optionally the contacting of step (b) is used for wound cleansing, wound bed preparation, to treat pressure ulcers, leg ulcers, burns, diabetic foot ulcers, scars, I.V. fixation, surgical wounds or minor wounds.

Claim 215 to 217 (canceled)

Claim 218 (previously presented): A medical dressing comprising a polypeptide having a protease activity, wherein the polypeptide comprises the polypeptide of claim 60.

Claim 219 (previously presented): An antitoxin composition comprising a polypeptide having a protease activity comprising the ability to hydrolyze a toxin, wherein the polypeptide comprises the polypeptide of claim 60.

Claim 220 (previously presented): A decontamination or anti-biological warfare agent comprising the polypeptide of claim 60, wherein the polypeptide having a protease activity has the ability to hydrolyze a toxin or an essential viral or microbial agent.

Claim 221 (previously presented): A medicament or pharmaceutical formulated for use in tissue dissociation, wound cleansing, wound bed preparation, treating pressure ulcers, treating leg ulcers, treating burns, treating diabetic foot treating ulcers, treating scars, intravenous (I.V.) fixation, or treating surgical wounds or minor wounds, wherein the medicament or pharmaceutical comprises the polypeptide of claim 60.

Claim 222 (canceled)

Claim 223 (currently amended): The isolated, synthetic or recombinant polypeptide of claim 60, wherein the polypeptide has at least ~~[[95%]]~~ 97% sequence identity to SEQ ID NO:42.

Claim 224 (previously presented): The isolated, synthetic or recombinant polypeptide of claim 60, wherein the polypeptide has at least 98% sequence identity to SEQ ID NO:42.

Claim 225 (previously presented): The isolated, synthetic or recombinant polypeptide of claim 60, wherein the polypeptide has the sequence of SEQ ID NO:42.

Claim 226 (previously presented): The isolated, synthetic or recombinant polypeptide of claim 60, wherein the protease activity further comprises catalyzing hydrolysis of peptide bonds; an endoprotease activity or an exoprotease activity; a proteinase activity or a peptidase activity; a carboxypeptidase activity; an aminopeptidase activity; a serine protease activity; a metalloprotease activity, a matrix metalloprotease activity or a collagenase activity; a cysteine protease activity; an aspartic protease activity; a chymotrypsin, a trypsin, an elastase, a kallikrein or a subtilisin activity; or a dipeptidylpeptidase activity.

Claim 227 (previously presented): The isolated, synthetic or recombinant polypeptide of claim 60, wherein the protease activity is thermostable or thermotolerant.

Claim 228 (previously presented): The isolated, synthetic or recombinant polypeptide of claim 60, wherein the polypeptide comprises at least one glycosylation site.

Claim 229 (previously presented): The isolated, synthetic or recombinant polypeptide of claim 60, wherein the polypeptide retains a protease activity under conditions comprising a range from pH 4.0 to pH 10.5.